

Application No. 10/539,212  
Response to Office Communication dated December 30, 2009

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Docket No.: 60364(71699)

### **REMARKS**

Claims 1 – 3, 6 – 9, 11 – 12 and 18 are pending in the application. Claims 5, 6, 10 and 13-17 have been previously cancelled. Claims 1, 6, 12 and 18 have been amended. No new claims have been added. No new matter has been added.

Any cancellation of the claims should in no way be construed as acquiescence to any of the Examiner's rejections and was done solely to expedite the prosecution of the application. Applicant reserves the right to pursue the claims as originally filed in this or a separate application(s).

#### **Claim Rejections 35 USC §112**

The Examiner has rejected claims 1 – 3, 6 – 9, 11 – 12 and 18 under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. (Office Action, p.4).

The Examiner argues that "(t)he claims are now directed to StxB1 B subunit (and) (t)his terminology is unclear because the specification refers to Stx1B." (Office Action, p.4).

Applicants have amended the claims to recite "Stx1B" as set forth in the specification. Applicants respectfully request that the rejection be withdrawn.

#### **Claim Rejections 35 USC §103(a)**

The Examiner has maintained the rejection of claims 1 – 3, 6 – 9, 11 – 12 and 18 under 35 USC §103(a) as being unpatentable over the combination of Marcato et al. (Infection and Immunity vol. 70 p.1279 (2002) in view of LaCasse et al. (Blood vol. 88 p.1561 (1995)) and Strockbine et al. (J Bacteriology vol. 170 p.1116), Accession Number 2002:397002, Green (US 2002/0081307) and Applicant's admission on page 6, lines 1 – 2 of the specification. (Office Action, p.3). Applicants respectfully disagree.

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The present claims recite a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B subunit of Shiga toxin.

As discussed in the previous response, Applicants have **particularly identified Stx1B for use in the methods as claimed**. Applicants teach that there are a number of Shiga toxin variants and subunits, for example at page 6, beginning at line 30 of the present disclosure:

The sequences of numerous Shiga toxin variants and subunits are known in the art. For example, the Shiga toxin 1 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400300 and 32400303, the Shiga toxin 2 B-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No. 13359150, the Shiga toxin 1 A-subunit is set forth from the E. coli O157:H7 strain is set forth in GenBank Accession Nos. 32400299 and 32400302, and the Shiga toxin 2 A-subunit from the E. coli O157:H7 strain is set forth in GenBank Accession No. 15718405.

Among all of these variants and subunits, **Applicants have particularly identified Stx1B for use in the methods as claimed**.

Further, Applicants demonstrate that StxB1 can be used for reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3. For example, in Example 7 beginning at page 43, Applicants show that Stx1B alone induces apoptosis in human colon cancer cells. In these experiments, Applicants show that "Stx1B internalization caused massive DNA fragmentation in Caco-2 cells, compared to intact non-fragmented DNA from cells not exposed to Stx1B (lane b) or incubated with CTB (Cholera Toxin B subunit) (lane e)."

In Example 8, on pages 43 - 44, Applicants show that Stx1B selectively causes apoptotic death in cells expressing Gb3.

In Example 10 beginning at page 45, Applicants use a nude mouse xenograft model to determine if Stx1B has apoptotic effects on growing tumors. Applicants show in Figure 8 that Stx1B injections significantly inhibited tumor growth in nude mice

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Marcato et al. teach the use of the cloned shiga toxin B (Stx2 B) subunit to induce apoptosis in Burkitt Lymphoma B-cells. Marcato et al. teach that **"apoptosis was not observed in A subunit-free preparations of the Stx1B pentamer."** (p.1279; emphasis added). Marcato et al. describes the apoptotic activity of the cloned Stx2 B subunit that, based on their results, "appears to be more potent in the Stx2 B than Stx1 B subunit." (p. 1279). Marcato et al. examine the apoptotic activity of Stx1 or Stx2 in Ramos Burkitt lymphoma B cells, and show that "(i)n contrast to the Stx2 B subunit, **the Stx1 B subunit did not induce apoptosis** in Ramos cells, even at the highest concentration." (P. 1280 – 1281 and Figure 1B; emphasis added). Further, referring to Fig. 1A, Marcato et al. show that Stx1 B does not induce apoptosis in Daudi cells. Marcato et al. use Z-VAD-fmk, a peptide that prevents caspase-mediated induction of apoptosis, to show that apoptosis induced by both the Stx1 or Stx 2 holotoxins or the cloned Stx2 B subunit was reduced, **but apoptosis was not reduced by Stx1B**. See Fig. 5A and p. 1281 – 1282. Marcato et al. show in Fig. 5B, that the **Stx1 B subunit does not exert a general antiapoptotic effect in Ramos cells** (see Fig. 5B, No treatment and Stx 1B compared to Camptothecin, an apoptosis inducer).

The Examiner alleges that "on page 1285, first full paragraph, the reference cites work by Nakagawa et al." and argues that "the results suggested that the Stx1B subunit must enter the cytoplasm of a eukaryotic cell to induce apoptosis." (Office Action, p.3). Referring to this reference in context, Marcato et al teach on p. 1285 that "the data presented in a report by Nakagawa suggest that apoptosis was not triggered in HeLa/C4 or NIH 3T3 cells exposed to Stx1 B subunit (and)...(a)poptosis was activated only when the cells were transfected with the *stxb1* gene, suggesting that the Stx1 B subunit must enter the cytoplasm to induce apoptosis." Further, Marcato et al. teach that "the data in our experiments allow us to come to similar conclusions as Nakagawa et al., because **excess Stx1 B subunit inhibited initiation of apoptosis** by both Stx 1 and Stx 2 holotoxins or the Stx2 B subunit, presumably by access to the Gb3-Cer receptors." (p.1285; see Fig. 5B). Marcato et al. are using the data of Nakagawa, where cells were transfected with *stxB1* gene in order to induce apoptosis, that is bypassing the normal endocytotic pathway of toxin internalization, to confirm their

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hypothesized mechanism of apoptotic death induced by Stx2 and Stx1 and the StxB2 subunit.

The teachings of Marcato would not lead one of skill in the art to choose StxB1 as an apoptosis inhibitor in the claimed methods.

Nowhere in the Marcato reference is there teaching or suggestion of a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B subunit of Shiga toxin as claimed.

None of the LaCasse, Strockbine or Greene references cure the defects of the Marcato reference. None of the references, alone or in combination, teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject, wherein the tumor cells produce Gb3, comprising administering to the subject a therapeutically effective amount of a Stx1B -subunit of Shiga toxin.

As previously discussed, the LaCasse reference is directed to the use of shiga like toxin (SLT-1) in human bone marrow (BM) purging. LaCasse uses Shiga Like Toxin (SLT-1) which kills cells by inhibiting protein synthesis. (p.1561). The purpose of the study described by LaCasse "was to establish the potential of a natural toxin (SLT-1) in purging B-cell lymphomas from BM." (p.1563).

LaCasse does not teach or suggest a method of reducing, or inhibiting invasiveness and metastasis of tumor cells in a subject using Stx1B.

The Examiner argues that "LaCasse et al disclose treatment of human B cell lymphoma from bone marrow in mice using Shiga-like toxin 1 (and) also discloses that the toxin was administered after the cancer is present." (Office Action, p.4). The Examiner argues further that "(o)n page 6 of the specification, Applicant admits the toxins are known to bind to Gb3 expressing cells, therefore it is expected that the cells of the reference are Gb3 expressing cells." (Office Action, p.4).

It would not have been obvious to one of ordinary skill in the art that StxB subunit can also be used to inhibit apoptosis in vivo, as argued by the Examiner on page 5 of the Office Action. The teachings of Marcato would not motivate one to use Stx1B in

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place of SLT-1, as taught by LaCasse. The teachings of the cited art, when combined, do not result in the claimed invention.

Accordingly, Applicants request that the rejection be withdrawn and the claims allowed.

### CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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